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RDT&E BUDGET ITEM JUSTIFICATION SHEET (R-2 Exhibit)								DATE February 2000	
APPROPRIATION/BUDGET ACTIVITY RDT&E, Defense-wide BA2 Applied Research					R-1 ITEM NOMENCLATURE Biological Warfare Defense PE 0602383E, R-1 #15				
COST (In Millions)	FY 1999	FY2000	FY2001	FY2002	FY2003	FY2004	FY2005	Cost To Complete	Total Cost
Total Program Element (PE) Cost	84.009	131.705	162.064	160.180	169.000	189.000	205.000	Continuing	Continuing
Biological Warfare Defense Program BW-01	84.009	131.705	162.064	160.180	169.000	189.000	205.000	Continuing	Continuing

(U) **Mission Description:**

(U) DARPA's Biological Warfare Defense program is budgeted in the Applied Research budget activity (BA-2) because its focus is on the underlying technologies associated with pathogen detection and remediation. Today, there is a tremendous mismatch between the magnitude of the biological warfare threat and the Department's ability to adequately respond. The widespread availability of bacterial, viral, toxin, and chemical stocks; minimal developmental cost and scientific expertise required; and abundance of weaponization potential comprises a sinister threat. The single largest concern, however, is from the exploitation of modern genetic engineering by adversaries to synthesize "super pathogens." Recent dramatic developments in biotechnology, which this program will leverage, promise to eliminate this mismatch. This program funds projects supporting revolutionary new approaches to biological warfare (BW) defense and does not duplicate efforts of other government organizations.

(U) Efforts to counter the BW threat include developing barriers to block entry of pathogens into the human body (including unique methods for rapid air and water purification), countermeasures to stop pathogen and chemical consequence and to modulate host immune response, medical diagnostics for the most virulent pathogens and their molecular mechanisms, biological and chemically-specific sensors, advanced decontamination and neutralization techniques, consequence management tools, and integrated defensive systems. Program development strategies include collaborations with pharmaceutical, biotechnology, government, and academic centers of excellence.

(U) Pathogen countermeasures (e.g., Anti-Virals/Immunizations, Anti-Bacterials/Anti-Toxins, Multi-Purpose, and External Protection) under development include: (1) multi-agent therapeutics against known, specific agents and (2) therapeutics against virulence pathways shared by broad classes of pathogens. Specific approaches include modified red blood cells to sequester and destroy pathogens or other toxic compounds, modified stem cells to detect pathogens and produce appropriate therapeutics within the body, identification of virulence mechanisms shared by pathogens, development of therapeutics targeting these mechanisms, efficacy testing in cell cultures and animals, and advanced non-toxic decontamination strategies.

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(U) In the early stages, many illnesses caused by BW agents have flu-like symptoms and are indistinguishable from non-BW related diseases. Early diagnosis is key to providing effective therapy. The advanced diagnostics efforts will develop the capability to detect the presence of infection by biological threat agents, differentiate them from other significant pathogens, and identify the pathogen even in the absence of recognizable signs and symptoms (when the pathogen numbers are still low).

(U) The ability to rapidly detect biological warfare agents on the battlefield with a low false-alarm rate is a crucial requirement. To address this need, the program is creating more efficient and effective miniature sampling technologies that concentrate contaminated air and enhance the ability to capture biological warfare agents. The program is developing a new range of antibodies and “designer small molecules” to bind specific agents (to replace the lower affinity antibodies currently used). In order to detect that the binding of an agent has occurred, the event must be “magnified.” Traditionally, this is done by tagging the antibody molecule with a fluorescent probe. This program is replacing the noise-plagued fluorescent tags with Up-Converting Phosphors with the sensitivity to detect a single binding event, minimizing the size of the sample required, saving time, and decreasing the number of false positive alarms. The use of fluids as a requirement for biological agent detection is also being eliminated and replaced by a miniaturized (shoe box-size) time-of-flight mass spectrometer. Development of a bacterial biochip to identify genus and species without multiplying the DNA by the polymerase chain reaction (PCR) is also under development, thereby saving at least 20 minutes in time to identification. Additional efforts are focusing on the construction of molecular, cellular, and multicellular sensors for the rapid detection of biological threats. These cellular and tissue-based sensors have the ability to respond to both known and unknown threats, determine live vs. inactivated threat status, and report functional consequences of exposure (mechanisms of action). The use of organisms such as insects are also being explored as information collectors for environmental biological or chemical threats. A variety of applications for these sensors are being explored including protection of buildings from a biowarfare agent attack.

(U) Mission effectiveness requires rapid, correct medical responses to biological weapon threats or attacks. This project will provide comprehensive protocols to protect or treat combatants by using current and emerging biological countermeasures. It will provide accelerated situational awareness for biological warfare events by detecting exposure to agents through an analysis of casualty electronic theater medical records and will locate and determine the most effective logistical support for providing appropriate treatment and pathogen-specific resources required to mitigate effects of the attack.

(U) DARPA is working with a number of governmental organizations to exploit recent advances in high throughput genetic sequencers to obtain complete genetic information on a number of important pathogens and their non-pathogenic nearest neighbors. This will allow us to develop

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an inventory of genes and proteins that distinguish pathogens from non-pathogens and to identify pathogenic markers in any guise. This information will be used to provide superior molecular targets and enable new generations of detectors, diagnostics, and therapeutics.

(U) DARPA is developing technologies for integrated defensive systems to be employed in buildings to protect inhabitants and enhance the capability to decontaminate exposed surfaces. In addition to advanced sensors, DARPA is pursuing low-pressure-drop filters, advanced decontamination and neutralization techniques, and fate and transport models to predict agent location and lethality.

(U) Lastly, DARPA is sponsoring a one-year investigation in FY 2000 of a technology that uses a new material (aerogel) for the collection of agents of biological origin. Aerogel is a term used to describe very low-density, highly porous, polymeric materials that provide a highly efficient, lightweight collection medium for airborne particles.

(U) **Program Accomplishments and Plans :**

(U) **FY 1999 Accomplishments:**

- Anti-Virals/Immunizations. (\$ 14.820 Million)
  - Developed a modified stem cell, which can both detect and produce a prophylactic/therapeutic response to a pathogen (in cell culture).
  - Determined (in-vitro) toxicity of modified stem cell-produced therapeutics.
  - Created techniques to rapidly develop immunization strategies against bacterial and viral pathogens and toxins.
- Anti-Bacterials/Anti-Toxins. (\$ 14.857 Million)
  - Developed and tested (in-vitro) cellular platforms for toxin destruction and toxin binding decoys.
  - Demonstrated selected strategies (in cell culture) to:
    - Inhibit the expression of disease causing (virulence) factors by pathogens.
    - Disrupt the disease causing (virulence) communications between pathogens.
    - Modulate the body's response to the presence of a pathogen.

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- Multi-Purpose. (\$ 12.000 Million)
  - Defined animal models in which to test the efficacy of modified stem cells to prevent disease.
  - Demonstrated in laboratory animals the efficacy of modified red blood cells to eliminate pathogens from the blood for the purpose of potential defense against biological warfare agents.
  - Determined pathogen detection and elimination efficacy for modified red blood cells with enzymes or other active molecules attached to their surfaces.
- External Protection. (\$ 6.450 Million)
  - Developed polymeric materials for pathogen protection.
  - Demonstrated in-vivo broad-spectrum efficacy of non-toxic biological decontamination formulation.
- Advanced Diagnostics. (\$ 10.900 Million)
  - Determined appropriate bodily sample types (blood, saliva, sputum, etc.) to use for diagnosis.
  - Determined which non-biological warfare (BW) pathogens must be screened against because they mimic early symptoms of known BW threat agents.
  - Began identification of probes to be used in diagnosis systems.
  - Evaluated the feasibility of novel technologies and sampling strategies, such as detecting bodily responses indicative of infection.
- Sensors. (\$ 15.390 Million)
  - Continued development of air sampling technology for airborne biological materials.
  - Determined chemotaxonomic biomarkers for selected viral substances for detection in a mass spectrometer.
  - Demonstrated replacement of a surface-bound antibody with a “designer” small molecule for high affinity pathogen capture.
  - Developed a high affinity monoclonal antibody that recognizes only anthrax spores without cross-reactivity with vegetative cells (or other bacillus species) and tested in existing BW sensors for improved performance.
  - Completed Up-Converting Phosphors (UCP) detection system and field test.
  - Modified the prototype of a miniature biodetection system following Dugway Proving Ground test results.
  - Selected cell and tissue types for the development of tissue based sensors.

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- Examined and selected strategies to stabilize cell systems for long-term shelf life and functional response.
- Demonstrated the ability to modify the duty cycle of a cellular response in single cell and tissue based sensors.
- Demonstrated performance limits of a single cell sensor.
- Consequence Management. (\$ 8.600 Million)
  - Developed prototype software toolkit for Enhanced Consequence Management Planning and Support System (ENCOMPASS).
  - Conducted field tests of BW defense attack response planning tools and Electronic Watchboard.
  - Developed Electronic Watchboard architecture and BW incident playbook authoring and maintenance tools.
  - Incorporated USAMRIID biological warfare agent treatment directives into playbooks and accelerated development of Biological Agent Symptom Information System (BASIS).
- Multimedia/Telemedicine. (\$ 0.992 Million)
  - Developed an enhanced telemedicine capability for the warfighter by augmenting/tailoring wireless communication technologies appropriate for responses to biological warfare attacks.

**(U) FY 2000 Plans:**

- Anti-Virals/Immunizations. (\$ 16.999 Million)
  - Identify broad-spectrum strategies with potential for immunomodulatory activity against multiple pathogens.
  - Develop technologies for rapid design and development of new vaccines against novel pathogens.
  - Demonstrate (in-vitro) candidate anti-viral small molecule therapeutics for selected targets.
  - Demonstrate (in-vivo) the efficacy of anti-viral peptides derived from hematopoietic stem cells.
- Anti-Bacterials/Anti-Toxins. (\$ 17.065 Million)
  - Develop (in-vitro) broad spectrum, superantigenic, anti-toxin antagonists and vaccines.
  - Validate the efficacy (in-vivo) of antagonists to toxin receptors, toxin catalytic sites, and cellular platforms for toxin destruction.
  - Demonstrate (in-vivo) the efficacy of a broad-spectrum bacterial antagonist.
  - Use gene-shuffling techniques to generate molecules to be screened for superantigenic properties.

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- Multi-Purpose. (\$ 16.466 Million)
  - Explore concepts for therapeutics against bioregulators and other mid-spectrum agents.
  - Identify primary harmful immune responses to biological warfare (BW) agents.
  - Explore concepts for optimizing human immune response to BW agents, minimizing negative sequelae.
  - Demonstrate in laboratory animal models the ability of modified stem cells to prevent disease.
  - Identify monomeric and dimeric DNA and RNA binding molecules as novel countermeasures against multiple pathogens.
  - Identify polyvalent inhibitors for inhibiting pathogens on the surface of target cells in-vivo.
- External Protection. (\$ 17.137 Million)
  - Develop decoy molecules that will prevent the adhesion of multiple pathogenic toxins or viruses in-vivo.
  - Demonstrate (in-vivo) a non-specific surfactant agent to neutralize biological threat agents.
  - Demonstrate initial performance of a prototype device for the purification of water contaminated with BW agent simulants.
  - Explore high throughput methods for the purification of contaminated air.
  - Demonstrate effectiveness of specific personnel protective toxin and pathogen neutralization strategies against virulent biological agents.
- Advanced Diagnostics. (\$ 15.792 Million)
  - Continue identification and development of probes to be used in diagnosis systems, and begin testing of probe panels in the laboratory.
  - Develop sample preparation techniques to optimize speed, accuracy, and reliability of diagnosis.
  - Identify one or more promising strategies for rapid detection based on bodily responses or other biomarkers (including cytokines) to provide early indication of infection or exposure (including non-invasive early detection of disease [e.g., nitric oxide in exhaled breath]).
  - Determine feasibility of engineering red blood cells to detect and signal pathogen presence in the body.
  - Determine feasibility of rapid single molecule DNA sequencing for accelerated patient diagnosis.
  - Explore concepts for diagnosing patients for bio-regulator and other mid-spectrum agent attack.
- Sensors. (\$ 27.746 Million)
  - Complete, test, and verify first-generation prototype of live agent biochip sensor.

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- Complete development of air sampling technology for airborne biological material.
- Continue development of effective and rapid chip-reading capability with enhanced sensitivity.
- Continue the development of unique signatures for bio-agents in mass spectrometry identification.
- Develop biosensor technology for next-generation (bioengineered) threat agents.
- Develop methods for identifying bioregulator-based BW agents.
- Explore options (e.g., training, genetic engineering, etc.) for the use of invertebrates in the detection of BW agents and associated chemicals.
- Construct cell and tissue engineered configurations to enhance optical or electrical signal output from the sensor.
- Investigate optimal system designs for deployment of a single cell and tissue based biosensor, which incorporate environmental sampling, microfluidics, and automated detection.
- Evaluate cell and tissue based informatics from temporal and spatial signals in cell and tissue-based sensors.
- Explore shelf-stabilization strategies for cells and tissues.
- Develop bio-agent sensors and other technologies for use in building protection (fate and transport).
- Develop the capability to predict flow of airborne bio-agents in and around buildings.
- Develop neutralization and decontamination techniques appropriate to buildings.
- Genetic Sequencing of Biological Warfare Agents. (\$ 4.000 Million)
  - Develop inventory of DoD-relevant BW agent pathogens requiring sequencing.
  - Determine best methods for rapidly sequencing biological warfare pathogens and related species and strains.
  - Begin development of database mining techniques to find new targets for sensors, diagnostics, and therapeutics.
- Consequence Management. (\$ 10.000 Million)
  - Develop distributed BW consequence management smart checklists for automatic pull and push of required information.
  - Continue development of Enhanced Consequence Management Planning and Support System (ENCOMPASS) software toolkit.
  - Demonstrate use of ENCOMPASS for OCONUS air base force protection against a BW attack.
  - Demonstrate use of playbooks and automated checklists for training BW incident responders.
  - Integrate Consequence Assessment Tool Set (CATS) with Electronic Watchboard using the ENCOMPASS architecture.

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- Asymmetrical Protocols for Biological Warfare Defense. (\$ 3.500 Million)
  - Initiate an effort in support of biological warfare defense against asymmetrical threats.
- Aerogel. (\$ 3.000 Million)
  - Investigate and test capture efficiency as a function of aerogel porosity and composition.
  - Develop aerogel coatings with greater flexibility and adherence to mass spectrometer tape.

**(U) FY 2001 Plans:**

- Anti-Virals/Immunizations. (\$ 21.300 Million)
  - Test and validate (in-vivo) a method of mucosal immunization based upon high level expression of pathogen antigens and epithelial transport molecules in edible transgenic plant products.
  - Test and validate (in-vivo) the protective efficacy of vaccines and antibodies produced by plant cells against pathogens.
  - Demonstrate efficacy of the rapid and efficient delivery of pathogen antigens via new genetic vaccine vectors.
  - Demonstrate (in-vivo) the rapid design and development of new vaccines (or therapeutics) against unidentified or unknown pathogens.
  - Demonstrate broad-spectrum strategies with potential for immunomodulatory activity against multiple pathogens.
- Anti-Bacterials/Anti-Toxins. (\$ 21.658 Million)
  - Demonstrate surface expression of specific enzyme molecules for the rapid inactivation of various pathogens.
  - Demonstrate (in-vivo) the efficacy of a broad-spectrum bacterial pathogen antagonist.
  - Validate (in-vivo) broad spectrum, superantigenic, anti-toxin antagonists and vaccines.
  - Demonstrate (in-vivo) efficacy of broad spectrum, superantigenic, antitoxin antagonists and vaccines.
- Multi-Purpose. (\$ 22.200 Million)
  - Develop therapeutic strategies against bioregulators and other mid-spectrum agents.
  - Demonstrate synthetic polymer complements for pathogenic antigens and virulence factors.
  - Develop therapeutic strategies for minimizing harmful immune responses to biological warfare agents.

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- Demonstrate (in-vitro) the efficacy of monomeric and dimeric DNA and RNA binding molecules as novel countermeasures against multiple pathogens.
  - Validate polyvalent inhibitors for blocking pathogens on the surface of target cells in-vivo.
  - Identify superantigens for broad protection against biological warfare agents with minimal side effects.
  - Validate (in-vivo) the efficacy of subcellular pathogen response imaging for rapid detection.
  - Validate technologies broadly applicable to enhance cellular therapeutics (delivery platforms) and virulence modulation (intracellular and inflammatory cascades).
- External Protection. (\$ 21.000 Million)
  - Develop a novel architectural approach for the manufacture of materials that are effective in blocking pathogens and limiting disease.
  - Demonstrate a non-aqueous advanced decontamination method.
  - Demonstrate a water purification system effective against a range of biological agents (including toxins and bioregulators).
  - Test initial performance of advanced sorbent materials for the purification of air contaminated with CW and BW agent simulants for individual protection.
  - Build and test a prototype air purification system for collective protection for a group of soldiers.
  - Begin testing of prototype protective system against non-virulent biological warfare agents, bio-toxins, and regulators.
- Advanced Diagnostics. (\$ 19.350 Million)
  - Test probe panels in relevant sample types including strategies for rapidly generating new/novel probes.
  - Demonstrate that sample collection and/or preparation techniques do not introduce artifacts.
  - Test, in model systems, one or more of the most promising candidate strategies for rapid detection based on bodily responses or other biomarkers to provide early indication of infection or exposure.
  - Develop the capability to diagnose exposure to bio-regulator and mid-spectrum agents.
  - Demonstrate, in the laboratory, the feasibility of engineering red blood cells to detect and signal pathogen presence in the body.
  - Evaluate the feasibility of additional strategies (e.g., exhaled breath) for direct identification or detection of infection without direct sample collection.
  - Evaluate instrument designs to perform accelerated patient diagnosis using a rapid single molecule DNA sequencing technique in a model system.

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- Sensors. (\$ 24.056 Million)
  - Develop effective and rapid chip-reading capability with enhanced sensitivity and low false alarm rate.
  - Develop advanced alternative technologies for live vs. dead bio-agent identification using peptides and other molecules.
  - Evaluate methods for removing micro-encapsulation of disguised pathogens and/or sensing through the micro-encapsulation.
  - Develop technologies required for next-generation miniature biological detectors including the use of microelectromechanical systems (MEMS), microfluidics, and mesoscopic-sized components.
  - Evaluate false positive and false negative rates for systems of detectors using biomolecular cells or tissues.
  - Exploit and/or mimic the olfactory sensors of biological systems for use in the detection of biological warfare agents.
  - Demonstrate enhanced signal output from engineered cells and tissue based sensors and integrate information from these sensors with user interfaces for predictive responses.
  - Engineer a deployable prototype cell and tissue sensor for field-testing.
  - Develop biosensor models and robust characterization protocols.
  - Investigate standoff techniques for trigger and identification.
  - Develop concepts for sensors capable of detecting biological warfare agent production in underground facilities.
  - Investigate critical design parameters for advanced biologically based biological warfare sensor.
  - Demonstrate use of organisms to collect chemical and biological warfare agents in the field.
- Bio/Chem Defensive Systems. (\$ 10.000 Million)
  - Continue fate and transport model development in and around buildings and begin experimental evaluation.
  - Continue to develop decontamination techniques appropriate for structures.
  - Evaluate novel low-pressure-drop, broadband filter technologies.
  - Develop neutralization technologies for aerosolized agents.
  - Conduct integrated system design and begin experimental evaluation.
- Genetic Sequencing of Biological Warfare Agents. (\$ 12.500 Million)
  - Continue the genomic sequencing of high-threat known and potential biowarfare agents.

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- Continue development of database mining techniques and test on a subset of pathogenic genomes.
- Consequence Management. (\$ 10.000 Million)
  - Demonstrate rapid construction and distribution of specific BW smart checklists for multiple responders.
  - Demonstrate Enhanced Consequence Management Planning and Support System (ENCOMPASS) management of multi-site BW incidents.
  - Demonstrate automatic construction of incident- and responder-specific playbooks and electronic watchboards.
  - Demonstrate use of ENCOMPASS for CONUS air base force protection against BW attacks.
  - Transition ENCOMPASS to National Guard Rapid Assessment and Initial Detection Units and to Air Force Theater Battle Management Core.

(U)	<b><u>Program Change Summary:</u></b> <i>(In Millions)</i>	<b><u>FY1999</u></b>	<b><u>FY 2000</u></b>	<b><u>FY 2001</u></b>
	Previous President's Budget	84.754	145.850	151.000
	Current Budget	84.009	131.705	162.064

(U) **Change Summary Explanation:**

FY 1999	Decrease reflects SBIR reprogramming and minor program repricing.
FY 2000	Decrease reflects the net effect of congressional program reductions; congressional adds for aerogel material and asymmetrical protocols; government-wide rescission, and minor program adjustments.
FY 2001	Increase reflects Departmental direction to continue the demonstration of complete genomic sequencing of high-threat known and potential biowarfare agents and expansion of on-going efforts under external protection and medical countermeasures.

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(U) **Other Program Funding Summary Cost:**

- Not Applicable.

(U) **Schedule Profile:**

- Not Applicable.

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